REMARKS

Claims 1 and 27-45 are pending in the case. Claims 1, 28, 35, 37, 40, and 44 are amended. Claims 1, 35, and 40 are amended to identify the hydrogenase as belonging to the green algae family or cyanobacteria family. This amendment is supported by Applicants disclosure at, for example, paragraphs [0036] and [0040]. Claims 1 and 40 are amended to point out that the identified amino acids to be substituted within the hydrogen channel are independently substituted with an amino acid having properties that limit O_2 diffusion through the channel while allowing H_2 diffusion out of the channel and to indicate that the substituted amino acid reduces the diameter of the channel. This amendment is supported by Applicants' specification at, for example, paragraphs [0045] and [0049]. Claims 1, 35, and 40 are amended to indicate that the aminoacids selected for substitution are "identified". This amendment is supported at least by Figure 2. Other amendments are made to the claims to correct obvious typographical errors. Applicants believe that no new material has been added.

Paragraphs [0012], [0013], [0021], [0030], [0039], [0044], [0047], [0062], [0067], and [0073] are amended. These amendments have been made to correct obvious typographical errors in the published document. These amendments do not add any new matter to the application or affect the claimed invention, and are supported by the text of the application as filed.

All references to the specification in this amendment refer to Application Publication No. 200610228774. Applicants respectfully request reconsideration of the application as follows:

I. Objections to Drawings

The Examiner objects to Figures 1a and 2 for illustrating sequences without sequence identifiers. Appropriate sequence identifiers are now provided in amended paragraphs [0012] and [0013] of the Brief Description of the Drawings.

II. Non-Compliance with Sequence Rules

The Examiner objects to paragraph [0073] for failing to include sequence identifiers for the primers. Paragraph [0073] is amended to include the sequence identifiers. Submitted concurrently with this amendment is an electronic copy of the SEQUENCE LISTING for the above-identified application submitted under 37 C.F.R. § 1.821(e) and a Statement to Support Filing and Submission. Applicants respectfully request that the electronic copy of the SEQUENCE LISTING be entered into the application. The electronic copy contains no new matter (37 C.F.R. § 1.821 (g)). Support for the electronic SEQUENCE LISTING is found in the Figures and disclosure of the application as filed.

Applicants do not believe a paper copy is required at this time. If the Examiner further requires a paper copy, please inform the undersigned.

III. Claim Rejections under 35 U.S.C. § 112

A. Rejection of Claims 29-34, 36, and 41-43 for Indefiniteness

The Examiner rejects claims 29-34, 36, and 43 as unclear for using the word "bulky" or phrase "bulky residue" to identify amino acids, and requests clarification. Five of the 20 naturally occurring amino acids are exemplary "bulky" amino acids. These amino acids are isoleucine, tryptophan, leucine, histidine, and phenylalanine, described as such due to the R-group that characterizes an amino acid which is, in these five amino acids, bulky. Other modified naturally occurring amino acids, non-naturally occurring amino acids, and/or synthetic amino acids can also have a bulky R-group and are contemplated herein. Applicants' specification includes a citation to *Stryer* (Biochemistry, Fourth Edition 1995 New York: Freeman, pages 17-44) where amino acid residue characterizations are described (see Applicants' specification at paragraph [0022]) (please inform the undersigned if a copy of these pages of the ***Styer*** reference is needed).

The Examiner rejects claims 41 and 42 as indefinite for reciting an average channel size with two numbers, and requests clarification. As Applicants point out at the end of paragraph [0045], an H_2-channel is in constant flux and for that reason the "diameter measurements are averages not meant to be held to a static standard". Also, throughout the length of a channel, the channel diameter varies as demonstrated in Example 1, especially paragraphs [0063] and [0065]. Thus, a range of average diameters is appropriate for describing a channel size.

B. Rejection of Claims 1 and 27-45 as Lacking Written Description Support

Claims 1 and 27-45 are rejected for lack of support by Applicants' disclosure. The Examiner asserts that the specification fails to disclose the structure of all the mutants or variants of all hydrogenase enzymes within the scope of the claimed genus. The Examiner further asserts that the genus is large and includes mutants and variants having different structures, and therefore, that the scope of the claims encompasses many structurally unrelated polypeptides. Applicants respectfully disagree.

Applicants have determined through careful analysis of the HydA1 H_2-channel sequence and structure, strategic amino acid residues that when replaced with bulky amino acids causes the effective channel diameter to decrease such that the channel prevents passage of O_2 through the channel. Testing their findings in *silico* demonstrated that individual mutations and

combined mutations reduced the average overall channel diameter. See Tables 1 and 2.

Applicants have fully described of two HydA1 H_2-channel mutants having V240W mutations. See paragraph [0068]. The success of the experiments described in Examples 2 and 3 show that the predicted mutants can limit O_2 inhibition. Applicants further describe suitable host cells that can express the oxygen resistant hydrogenase, including green algae and cyanobacteria. See paragraph [0057].

Applicants have identified common characteristics of the claimed molecules, including structure, physical and/or chemical characteristics, and functional characteristics. The computer modeling of the hydrogenase H_2-channel revealed a hydrophobic channel extending from the active site to the enzyme surface, a channel that is conserved in other [Fe]-hydrogenases. The channel has a helical secondary structure. The channel diameter is sufficient to allow for the diffusion of H_2 as well as the inhibitors O_2 and CO. See paragraph [0067]. Applicants' modification of the channel to reduce overall channel diameter results in an improved enzyme which functions to produce hydrogen in a more efficient manner relative to the hydrogen channel of an oxygen-sensitive iron hydrogenase. See paragraphs [0076] and [0077] and Figs. 6 and 7.

Applicants cite and incorporate by reference literature publications characterizing oxygen-sensitive hydrogenases. See paragraph [0005]. Between family members, the sequence identity for [Fe]-hydrogenase enzymes is at least 45% and sequence identity for the hydrogen channel is at least 66%. See paragraph [0036]. Applicants further describe [Fe]-hydrogenase enzymes in paragraph [0038]. Oxygen-resistant hydrogenases conceived by Applicants have characteristics like the oxygen-sensitive hydrogenases, but the former have smaller channel diameters and are more efficient in producing H_2. See, for example, paragraph [0008].

Applicants have fully described the subject matter of the claims in the disclosure of the application, and therefore demonstrate possession of the claimed invention at the time the application was filed.

C. Rejection of Claims 1 and 27-45 for Non-Enablement

A patent application must be written such that one skilled in the art to which it pertains is enabled to make and use the invention without undue experimentation (In re Wands, 858 F.2d 731,737 (Fed. Cir. 1988)). A specification is presumed enabling (In re Marzocchi, 169 U.S.P.Q. 267,369 (C.C.P.A. 1971)), and may be so even though experimentation is required (*U.S.* v. Telectronics, *Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988)). The fact that experimentation may be complex does not necessarily make it undue if such experimentation is typically performed in the art (In re Wands, 858 F.2d at 737). The test is not whether any experimentation is necessary, but

whether necessary experimentation is undue (In re Angstadt, 537 F.2d 498, 504 (C.C.P.A. 1976).

As the Examiner points out, the relevant factors to be considered in making a determination of whether undue experimentation is required include, but are not limited to: (a) the breadth of the claims; (b) the nature of the invention; (c) the state of the prior art; (d) the level of one of ordinary skill; (e) the level of predictability in the art; (f) the amount of direction provided by the inventor; (g) the existence of working examples; and (h) the quantity of experimentation needed to make or use the invention based on the content of the disclosure (In re Wands, 858 F.2d at 737).

The Examiner rejects claims 1 and 27-45 for lack of enablement. Specifically, the Examiner asserts that the scope of the claims is large regarding the number of oxygen-resistant iron hydrogenases and the number of amino acids that can be substituted; the structural elements required in any oxygen-resistant iron hydrogenase derived from any oxygen sensitive hydrogenase are lacking; guidance is lacking that would show the replacement of one or more amino acid residues within the hydrogen channel with any or all amino acids will produce a variant with the desired biological characteristics; the art fails to provide insight regarding the changing of an amino acid residue of the hydrogen channel to produce an oxygen resistant hydrogenase; the art provides no information as to how the structure of the hydrogenase protein correlates with HydA1 protein or corresponding mutants nor the degree of structural similarity among all HydA1 proteins.

The claims as amended are fully enabled by the specification. Claims 1, 35, and 40 are amendkd to identify the hydrogenase as a green algae or cyanobacteria iron hydrogenase. Figure 2 shows the amino acid sequence of the HydA1 protein aligned to the catalytic core region of CpI. Sequences forming the H_2-channel domain are shaded either gray or black. Applicants' claims are directed to substitution of identified amino acid residues within the hydrogen channel, not any amino acid. The one or more identified amino acid residues are independently substituted with an amino acid having properties that limit O_2 diffusion through the channel while allowing H_2 diffusion out of the channel. Not just any amino acid can be used to replace an identified amino acid within the hydrogen channel. A replacement amino acid must be one that constructs an oxygen-resistant iron hydrogenase from an oxygen-sensitive hydrogenase as required by claims 1, 35, and 40. Such an amino acid is exemplified by tryptophan, isoleucine, leucine, histidine and phenylalanine, and can be a synthetic or derivatized amino acid that has similar properties. Thus, the breadth of the claims is enabled by the specification. See paragraph [0500] and Tables 1 and 2.

Applicants also provide a considerable amount of guidance to enable one of skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the amended claims. An oxygen-resistant hydrogenase functions to produce hydrogen in a manner more efficient than an oxygen-sensitive hydrogenase. This is due to the substitution of an amino acid within the hydrogen channel for a bulky amino acid. Thus, the change in structure correlates with the efficiency in which the hydrogenase functions. In paragraph [0037], Applicants characterize oxygen-sensitive hydrogenases, the catalytic core, and the H_2-channel. Then, in paragraph [0038], Applicants show how the oxygen-sensitive hydrogenase is modified to obtain the oxygen-resistant hydrogenase, an enzyme with the characteristics of the oxygen sensitive hydrogenase, but more efficient in producing hydrogen due to the decreased H_2-channel diameter.

Further, Applicants demonstrate in detail how they designed and engineered oxygen-resistant hydrogenases in paragraphs [0044] through [0048]. This description shows how homology modeling was used to identify amino acids within the hydrogen channel that when replaced, would generate a hydrogenase with decreased H_2-channel diameter. Desirable channel sizes are included, for example, between approximately 5.0 and 2.4 A. *See* paragraph [0045].

Through the use of computer modeling, Applicants devised an approach for mitigating the unpredictability in designing an oxygen-resistant hydrogenase. The substitution of any amino acid within the H_2-channel with any other amino acid would indeed produce unpredictable results and require undue experimentation. However, Applicants show that the targeted substitution of particular amino acids within the H_2-channel with bulky or hydrophobic amino acids produces a hydrogenase with a more predictable H_2-channel environment. *See* Examples 1 and 3. Thus, the art is not highly unpredictable such that one of skill would be able to, without undue experimentation, make or use the oxygen-resistant hydrogenase of the claims.

Example 1 demonstrates the use of computer modeling to provide approximate HydA1 structure and H_2-channel environment. When a selected amino acid is replaced with a bulky amino acid, the modeling software predicts the effects on the channel environment and diameter. See Figures 2 and 3, and Tables 1 and 2, as well as paragraphs [0063]-[0067]. Example 2 explains how Applicants generated two strains of C. *reinhardtii* mutants with oxygen-resistant hydrogenases, including growing conditions for the organism, plasmid constructs, mutagenesis techniques, and methods of transformation. In Example 3, Applicants describe how the mutants were tested for ability to produce hydrogen in the presence of oxygen, and include data verifying that the predicted amino acid substitution would produce an oxygen-resistant hydrogenase.

Thus, Applicants' description and the information provided in the Examples is more than sufficient to allow one skilled in the art to make and use oxygen-resistant hydrogenases within the scope of the claims. The breadth of the claims is commensurate with the disclosure, and the unpredictability of the art is mitigated by Applicants' experimental design. Therefore, the specification does enable one of skill in the art to make and use the claimed oxygen-resistant hydrogenase.

IV. **Claim Rejections under 35 U.S.C. § 102(e)**

The Examiner rejects claims 1, 27-29, and 31-45 as anticipated by Dillon in U.S. Publication No. 200710009942.

Dillon is allegedly directed to a method for evolving an iron hydrogenase by substituting any amino acid within the sequence $FX^1X^2X^3G^1G^2VMEA^1A^2X^4R$ of an iron hydrogenase with any amino acid, transforming an organism with a nucleic acid encoding the modified iron hydrogenase, then screening the organism for the ability to produce hydrogen in the presence of oxygen.

Applicants' claim 1 is directed to an oxygen resistant iron hydrogenase derived from green algae or cyanobacteria by substituting one or more identified amino acid residues within a hydrogen channel. The one or more identified amino acid residues are independently substituted with an amino acid having properties that limit O_2 diffusion through the channel by reducing the diameter of the channel while allowing H_2 diffusion out of the channel. Applicants have determined through careful analysis of the HydA1 H_2-channel sequence and structure, strategic amino acid residues that when replaced with bulky amino acids causes the effective channel diameter to decrease such that the channel prevents passage of O_2 through the channel. Testing their findings in *silico* demonstrated that individual mutations and combined mutations reduced the average overall channel diameter. See Tables 1 and 2. Dillon does not teach or suggest the targeted substitution of an identified amino acid with an amino acid that limits O_2 diffusion through the channel while allowing H_2 diffusion out of the channel. Thus, for at least this reason, Dillon fails to anticipate claim 1.

Claims 27-34 directly or indirectly depend from claim 1, and are therefore unanticipated for at least the same reasons as described above with respect to claim 1.

Applicants' claim 35 is directed to an oxygen resistant iron hydrogenase from green algae or cyanobacteria. The hydrogen channel of this hydrogenase is defined by one or more identified diameter determining amino acid residues which reduces the diffusion of oxygen within the channel relative to the diffusion of oxygen in the hydrogen channel of an oxygen-sensitive iron

hydrogenase. Dillon does not teach or suggest a hydrogen channel having a diameter defined by one or more identified diameter determining amino acid residues. Thus, for at least this reason, Dillon fails to anticipate claim 35.

Claims 36-39 are dependent on claim 35, and are therefore unanticipated for at least the same reasons as described above with respect to claim 35.

Applicants' claim 40 is directed to an oxygen resistant hydrogenase from green algae or cyanobacteria derived from an oxygen sensitive iron hydrogenase. One or more identified amino acid residues within the hydrogen channel of the oxygen resistant hydrogenase are substituted to reduce the oxygen sensitivity, and are independently substituted with an amino acid having properties that limit O_2 diffusion through the channel while allowing H_2 diffusion out of the channel. As pointed out above, Applicants have determined through careful analysis of the HydA1 H_2-channel sequence and structure, strategic amino acid residues that when replaced with bulky amino acids causes the effective channel diameter to decrease such that the channel prevents passage of O_2 through the channel. Testing their findings in *silico* demonstrated that individual mutations and combined mutations reduced the average overall channel diameter. See Tables 1 and 2. Also discussed above, Dillon neither teaches nor suggests the targeted substitution of an identified amino acid with an amino acid that limits O_2 diffusion through the channel while allowing H_2 diffusion out of the channel. Thus, for at least this reason, Dillon fails to anticipate claim 40.

Claims 41-45 are dependent on claim 40, and are therefore unanticipated for at least the same reasons as described above with respect to claim 40.

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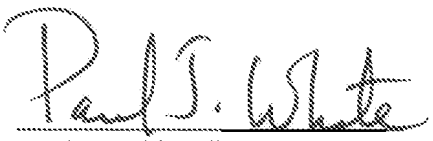
For the reasons set forth above, Applicants respectfully submit that the claims are allowable and reconsideration and issuance of a notice of allowance are respectfully requested. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 14-0460 if not otherwise specifically requested. The undersigned hereby authorizes the charge of any required fees not included or any deficiency of fees submitted herewith to be charged to deposit account No. 14-0460.

Respectfully submitted,



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